Table 1. Summary of *Tma* endonuclease V cleavage of heteroduplexed synthetic substrates containing single base mismatches.

Base change (Wt↔Mt)	A↔G	C↔T	A↔C	G↔T	A↔T	G↔C
Heteroduplex I:						
UpperStrand (Wt)	A +++	C +	A ++	G ++	A +++	G +++
BottomStrand(Mt)	C -	A ++	G +++	A ++	A +++	G +++
Heteroduplex II:						
UpperStrand (Mt)	G ++	T ++	C +	T ++	T +	C -
BottomStrand (Wt)	T ++	G +++	T ++	C -	T +	C -

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Note:
UpperStrand:
5'-FAM-TA CCC CAG CGT CTG CGG TGT TGC GTN AGT TGT CAT AGT TTG ATC CTC TAG TCT TGT TGC GGG TTCC-3' (SEQ. ID. No. 5)

BottomStrand:
3'- GGG GTC GCA GAC GCC ACA ACG CAN TCA ACA GTA TCA AAC TAG GAG ATC AGA ACA ACG CCC-TET-5' (SEQ. ID. No. 6)

Cleavage symbols:
(+++): high intensity cleavage. (++): intermediate intensity cleavage. (+): low intensity cleavage. (-) no cleavage

The cleavage of these heteroduplexed products are not always identical (i.e. compare A-C with C-A), and this reflects subtleties in the structure of the DNA as a consequence of neighboring sequence variation. Nevertheless, for each possible base change, signal is generated for at least one top strand and at least one bottom strand. Thus, the *Tma* EndoV enzyme should be able to recognize any possible single base mutation or polymorphism.

Please replace the paragraph beginning at page 74, line 11 with the following paragraph:

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Residues that are involved directly in protein-substrate interactions have a strong tendency to be conserved among enzymes of the same family. Therefore, in a primary amino acid sequence alignment, highly conserved residues represent good candidates for mutagenesis. In order to identify positions in *Thermotoga maritima* Endo V suitable for mutagenesis, the ClustalW alignment algorithm with a PAM250 Residue Weight

Table(Pairwise Alignment Parameters: Ktuple = 1, Gap Penalty = 3, Window = 5, and Diagonals Saved = 5; Multiple Alignment Parameters: Gap Penalty = 10 and Gap Length Penalty = 10) was used to perform a primary amino acid sequence alignment among 13 identified and putative Endo V enzymes from thermophilic and mesophilic archeabacteria and eubacteria (i.e. *Thermotoga maritima* (SEQ. ID. No. 37), *Pyrobaculum aerophilum* (SEQ. ID. No. 38), *Pyrococcus horikoshii* (SEQ. ID. No 39), *Pyrococcus abyssi* (SEQ. ID. No. 40), *Pyrococcus furiosus* (SEQ. ID. No. 41), *Archaeoglobus fulgidus* (SEQ. ID. No. 42), *Aeropyrum pernix* (SEQ. ID. No. 43), *Clostridium acetobutylicum* (SEQ. ID. No. 44), *Yersinia pestis* (SEQ. ID. No. 45), *Escherichia coli* (SEQ. ID. No. 46), *Bacillus subtilis* (SEQ. ID. No. 47), *Salmonella typhimurium* (SEQ. ID. No. 48), and *Streptomyces coelicolor* (SEQ. ID. No. 49), majority sequence, top line (SEQ. ID. No. 50) (Figure 19)). Since the majority of enzymes utilized in the alignment are putative Endo V enzymes, the mismatch specificity of most of these enzymes is unknown. As a result, when utilizing the alignment to identify candidate residues in *Thermotoga maritima* Endo V, one can either assume that the homologous enzymes have similar or different specificities. If one assumes similar specificities, then residues that are highly conserved for the majority of Endo V provides candidates. Whereas if one assumes different specificities, then positions where there exists two sets of highly conserved residues represent candidates.

A2 Cont.

Please replace the paragraph beginning at page 83, line 29, with the following paragraph:

The fluorescence labeled deoxyoligonucleotide substrates were prepared as described (Huang, J., et al., Biochemistry 40(30):8738-8748 (2001), which is hereby incorporated by reference in its entirety). The sequence of a typical inosine substrate (SEQ. ID. Nos. 1-2, respectively) is as follows:

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                                        ↓
5'-Pam-TA CCC CAG CGT CTG CGG TGT TGC GT   I AGT TGT CAT AGT TTG ATC CTC TAG TCT TGT TGC GGG TTC C-3'
      3'- GGG GTC GCA GAC GCC ACA ACG CA   I TCA ACA GTA TCA AAC TAG GAG ATC AGA ACA ACG CCC-Tet-5'
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A nick event at the top strand generates a 27 nt labeled product while that at the bottom strand generates a 38 nt labeled product. The cleavage reactions were performed at 65°C for 30 minute in a 20 µl reaction mixture containing 10 mM HEPES (pH 7.4), 1 mM DTT, 2% glycerol, 5 mM $MgCl_2$ unless otherwise specified, 10 nM DNA substrate, indicated amount

of purified Tma endonuclease V protein. The reaction was terminated by adding an equal volume of GeneScan stop solution. The reaction mixtures were then heated at 94°C for 2 min and cooled on ice. Three microliter of samples were loaded onto a 10% GeneScan denaturing polyacrylamide gel (Perkin Elmer). Electrophoresis was conducted at 1500 voltage for 1 hr using an ABI 377 sequencer (Perkin Elmer). Cleavage products and remaining substrates quantified using the GeneScan analysis software versions 2.1 or 3.0.

A3
Cont.